Identification of Sources of Resistance to Bacterial Angular Leafspot Disease of Strawberry

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Abstract

Bacterial angular leafspot disease (BALD) of cultivated strawberry, caused by the bacterium *Xanthomonas fragariae,* has become an increasingly serious disease problem. It is of particular concern because it is readily transmitted through asymptomatic nursery plants. Until now, there have been no sources of resistance to this pathogen identified in either commercial varieties or germplasm. We have used four genetically distinct strains of the pathogen, *Xanthomonas fragariae,* to screen 81 *Fragaria* genotypes, including both diploid and octoploid accessions, for resistance to this pathogen. Two genotypes, a native *F. virginiana* from Minnesota and a hybrid between a *F. virginiana* from Georgia and *F. ×ananassa* 'Earliglow', were found to be resistant to all four genotypes of this pathogen after leaf infiltration assays. Following infiltration of these genotypes, symptoms of the disease, including localized necrosis, leaf collapse, bacterial ooze production or systemic spread of the pathogen, were not observed. Plants of 'Sweet Charlie', used as the susceptible standard, showed all of these symptoms. The two resistant genotypes, designated US 4808 and US 4809 have been made available to the public as germplasm releases. Controlled crosses were made between the susceptible variety 'Sweet Charlie' and the two resistant genotypes. Resistance to *X. fragariae* was transmitted to 8-12% of the progeny of the US 4808 cross and to 4-18% of the progeny of the US 4809 cross. Data from these experiments are being analyzed to establish the mode of inheritance. Our research may lead to sustainable control of this disease.

INTRODUCTION

Bacterial angular leafspot disease of strawberry, (*Fragaria ×ananassa* Duch.) has become a chronic and at times serious disease of strawberries. The disease and the pathogen, *Xanthomonas fragariae* were originally described from Minnesota (Kennedy and King, 1962), but are now known to have a much wider distribution in North America as well as in Europe, South America and Australia (Maas et al., 1995). The primary symptom of the disease is translucent leafspots that are delimited by the veins of the leaf. Pale, white bacterial exudate is commonly observed on the underside of symptomatic leaves under conditions of high humidity, and these bacteria promote secondary spread of the disease in contaminated fields. Although the pathogen is not known to infect strawberry fruit, it is able to invade the plant systemically through the vascular system, and colonize the petioles, crown, stolons and roots (Milholland et al., 1996). Young plants may be entirely free of symptoms, and the pathogen can be disseminated with commercial transplants, which succumb to the disease in the field.

All commercial cultivars of strawberry are apparently susceptible to the pathogen, and prior to the initiation of this research, there were no sources of resistance to *X. fragariae* available to strawberry breeders. In order to identify germplasm resistant to the disease, we assembled a diverse collection of *Fragariae* germplasm (Maas et al., 2000) as well as a collection of strains of *X. fragariae* isolated from North America and Europe (Pooler et al., 1996). We characterized the population structure of the pathogen and developed an advance polymerase chain reaction (PCR)-based assay for the pathogen in plant tissue (Hartung and Pooler, 1997; Pooler et al., 1996). In the course of this work, we identified four genotypes of the pathogen, and have used these genotypes to screen our

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collection of *Fragaria* germplasm (Maas et al., 2000). We have identified two octoploid strawberry genotypes, US 4808 (a wild *F. virginiana* from Minnesota) and US 4809 (derived from a cross between *F. virginiana* SG26 and *F. ×ananassa* 'Earliglow') (Maas et al., 2002), that are very resistant to infection by *X. fragariae* after inoculation by leaf infiltration. We now report the intitial results of experiments to characterize the nature of the resistance to *X. fragariae*, which is heritable, observed in these octoploid *Fragaria* genotypes.

MATERIALS AND METHODS

Plant Material

Octoploid *Fragaria* clones US 4808 and US4809 (Maas et al., 2002) were maintained under greenhouse conditions and used separately as parents in crosses to the susceptible strawberry 'Sweet Charlie'. Seedling progeny (~ 100 per cross) from each of the two crosses were propagated by stolon-tip plantlet production. These progeny, as well as propagants from the original parental clones, were inoculated with *X. fragaria* as described below.

Bacterial Isolates and Inoculation Methods

X. fragariae strains ATCC33239, Xf-3, Xf-6 and Xf-1425 were selected because they represented the genetic variability present in a collection of strains of *X. fragariae* isolated from the US and Europe (Pooler et al., 1996). Bacteria were maintained as frozen glycerol stocks at –20^{o}C and then grown on sucrose peptone agar medium (Hayward, 1960) for three days at 27^{o} C. Bacteria were suspended in sterile deionized water and adjusted turbidimetrically to OD_{595} = 0.1 (≈ 10^{8} CFU/mL). Plants were watered and the leaves to be inoculated were labeled with tape prior to inoculation. The youngest, fully expanded leaf of each plant was selected for inoculation. The bacterial inoculum was drawn into a 3 mL syringe without the needle. The syringe was pressed against the lower leaf surface, while the plunger was depressed, to deliver the inoculum into the leaf mesophyll. Each leaflet was inoculated at four sites, for a total of 12 inoculation sites per leaf. After the inoculations, the plants were incubated in conditions favorable for disease development. The inoculated plants were enclosed in plastic bags for 3 days, and then set on a mist bench for one week, before being placed on a greenhouse bench. Separate experiments were done for each strain of the pathogen. Inoculations were replicated in each experiment so that 3 plants were inoculated with each strain of the pathogen.

Evaluation of Disease Symptoms

Plants were evaluated for symptoms at the end of the first week and weekly thereafter using a scale of 0-5 applied to each inoculation site (0 = no reaction (most resistant) and 5 = spreading necrosis (most susceptible))(Maas et al., 2000). The entire experiment was repeated three times, and each combination of strawberry population and bacterial strain was submitted to a separate ANOVA and Chi-square analysis to determine goodness of fit to various models of inheritance.

Bacterial Growth *in Planta*

Plants of US 4808, US 4809 and Sweet Charlie were inoculated as described above. Leaflets were taken after 17 days in the greenhouse. A sterile cork borer was used to excise the region surrounding the inoculation site, and each leaf disk was ground in sterile deionized water and ten-fold dilution series made in water were plated onto sucrose peptone agar using a spiral plater (Spiral Biotech). Plates were incubated for 4 days at 27^{o}C, and the colonies were counted.

RESULTS AND DISCUSSION

The inoculation method used enabled us to score the progeny accurately using the scale 0-5 as illustrated (Fig. 1). These quantitative evaluations of the relative susceptibility or resistance of a given strawberry genotype were reproducible across the exper-

iments, and the pooled data across strains and genotypes indicated that the broad sense heritability for resistance to *X. fragariae* was 96%. Depending on the strain of *X. fragariae* used, resistance was transmitted to 8-12% of the progeny of the US4808 cross and to 4-18% of the progeny of the US4809 cross. This shows that germplasm releases US 4808 and US 4809 will be very useful sources of resistance to *X. fragariae*.

Pathovars of the related taxon *Xanthomonas campestris* generally interact with their plant hosts based on a gene-for-gene model, where plant host resistance is conditioned by a dominant resistance gene that interacts with an avirulence gene in the bacterium to result in a resistant, hypersensitive interaction. In such a case, after infiltration of leaf mesophyll with bacteria at 10^8 CFU/mL, necrosis at the point of inoculation is observed within 18 hours after the inoculation, followed by a decline in bacterial population. Our inheritance data do not fit this gene-for-gene model, but instead indicate that there are likely to be three to four recessive plant genes that interact to condition a resistance response. An exception to the gene-for-gene model of disease resistance has recently been described for the interaction between pepper and *Xanthomonas campestris* pv. vesicatoria (Jones et al., 2002). In that case the authors did not observe hypersensitive necrosis, and the bacterial population did not fall off rapidly at the inoculation site in resistant plants, but instead increased over time in the absence of symptoms. In that case, two recessive resistance genes were defined in the host.

US4808 and US 4809 are clearly resistant to *Xanthomonas fragariae* after inoculation by leaf infiltration, because the pathogen is not able to induce disease symptoms in the host. However, a hypersensitive response was not observed. We have also observed in a preliminary experiment that populations of *X. fragariae* did not decline at the site of inoculation following infiltration, as might be expected. Instead the population at the inoculation sites of susceptible 'Sweet Charlie' and resistant US4808 and US 4809 were comparable 17 days post inoculation, at about 370,000 CFU per site. The interaction between *Fragaria* spp. and *Xanthomonas fragaria* may be similar to the recent example from pepper and *Xanthomonas campestris* pv. vesicatoria cited above, and is not typical of interactions between pathovars of *Xanthomonas campestris* and their plant hosts. We are in the process of thoroughly characterizing this interesting plant/ pathogen interaction. We are also in the process of mapping the resistance genes in the *Fragaria* genome. US4808 and US4809 have been made publicly available as germplasm releases (Maas et al., 2002).

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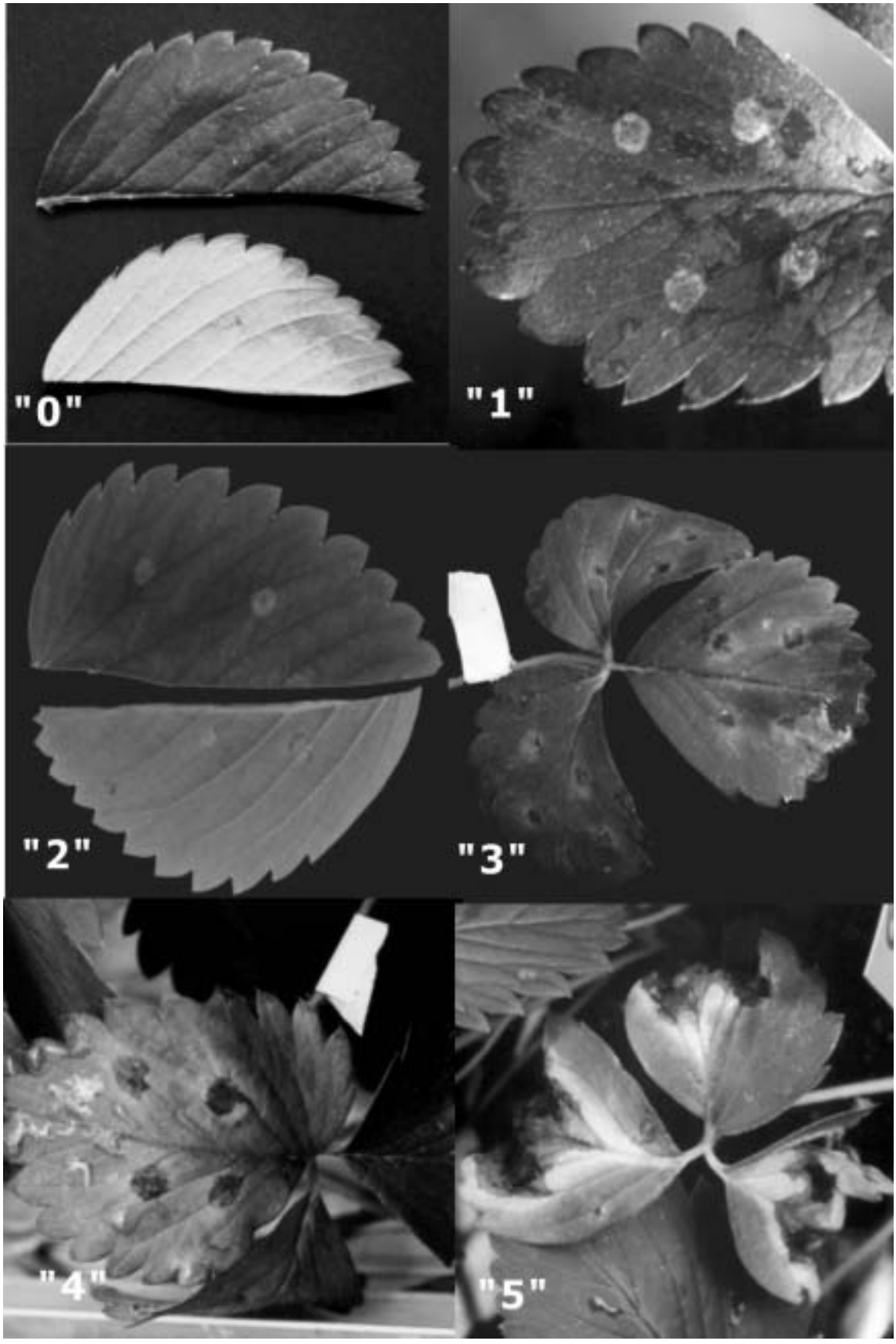
Fig. 1. Responses of *Fragaria* leaflets to infiltration with *Xanthomonas fragariae*. The photos illustrate the scale developed to rate parents and progeny of *Fragaria* for resistance to *X. fragariae*. "0", no reaction; "1" chlorosis but not necrosis; "2" chlorosis and necrosis limited to the inoculation site; "3" necrotic lesions limited to the inoculation site surrounded by chlorotic haloes; "4" necrotic lesions with chlorotic haloes expanding slightly beyond the inoculation site; "5" large spreading necrotic lesions with typical angular leafspot appearance and systemic movement.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